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ANIMAL REPRODUCTION

Evaluation of seasonal breeding of the domestic canary (*Serinus canaria*) in an artificial environment

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ABSTRACT: *The domestic canary (Serinus canaria) has been bred for hundreds of years to improve the quality of its plumage and its song. Reproduction in this species occurs seasonally, stimulated by a gradual increase in day length. Although, the occurrence of seasonal breeding in canaries is well known, whether canary reproduction can be manipulated remains unknown. Our objective was to determine whether the conditions of captivity (photoperiod and temperature) can be adjusted to enable canaries to reproduce outside of their natural breeding season. Thirty days before the natural breeding season, canary pairs were assigned and separated into three different groups: External Control (housed outdoors under ambient conditions), Artificial Control (housed artificially indoors under conditions similar to the external conditions), and Artificial Altered (housed artificially indoors for five months, with the photoperiod gradually manipulated to simulate that of the natural breeding season) groups. The number of clutches laid was greater in the Artificial Control than in External Control; however, more birds hatched in the External Control. In the Artificial Altered group, the beginning of the breeding season was delayed when the same parameters were used. Although, further research is needed, this study presents new data to assist in the development of protocols that entail gradual changes in environmental conditions to try to reduce the impacts of the processes on animal welfare.*
Key words: *Serinus canaria, chronobiology, photoperiod, reproduction, hatching.*

Avaliação da reprodução sazonal no canário doméstico (*Serinus canaria*) em ambiente artificial

RESUMO: *O canário doméstico (Serinus canaria) tem sido criado há centenas de anos para melhorar a qualidade da plumagem e do canto. A reprodução nesta espécie é sazonal, sendo estimulada por um aumento gradual da duração do dia. Embora a reprodução sazonal de canários seja bem conhecida, ainda não se sabe se a reprodução dos canários pode ser manipulada. O objetivo foi determinar se as condições em cativeiro (fotoperíodo e temperatura) podem ser ajustadas para permitir que os canários se reproduzam fora de sua estação natural de reprodução. Trinta dias antes da estação natural de reprodução, os pares foram designados e separados em grupos: Controle Externo (alojado em gaiolas sob condições ambientais externas); Controle Artificial (alojado em gaiolas mantidas em condições artificiais semelhantes ao lado externo); e Alterado Artificial (alojado em gaiolas mantidas em condições artificiais constantes por cinco meses, após o fotoperíodo foi gradualmente manipulado para simular o da estação natural de reprodução). O número de posturas foi maior no grupo Controle Artificial do que no Controle Externo; entretanto, mais aves eclodiram no Controle Externo. No grupo Alterado Artificial, o início do período reprodutivo foi atrasado considerando os mesmos parâmetros reprodutivos. Embora mais pesquisas sejam necessárias, este estudo apresenta novos dados para auxiliar no desenvolvimento de protocolos que promovam mudanças graduais das condições ambientais visando reduzir os impactos no bem-estar animal.*
Palavras-chave: *Serinus canaria, cronobiologia, fotoperíodo, reprodução, incubação.*

INTRODUCTION

The canary (*Serinus canaria*) is a small bird that nests in shrubs in nature. It has an attractive plumage of a yellowish-green and light grey, with long wings and various shades. Its song is loud and sonorous. This species has given rise to all currently known breeds and varities of canaries (BIRKHEAD et al., 2004). The first reports of the introduction of this bird to new regions are from the fifteenth century, when Portuguese and Spanish navigators brought canaries from the Canary Islands to Central Europe (PARSONS, 1987). Selective breeding of the canary has resulted in more than 400 different colours in this species of important economic interest (CANTONI, 2014).

Canaries are photosensitive and exhibit reproductive neuroendocrine responses to environmental factors such as light (photoperiod).

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Light stimulates the secretion of gonadotrophin releasing hormone (GnRH) and is essential for the consolidation of breeding periods in birds (STOREY & NICHOLLS, 1976). Under natural conditions, the onset of egg laying in females of various bird species can be advanced or delayed by photoperiod manipulation (BENTLEY et al., 2003; MEIJER et al., 1999).

Biological rhythms are observed in almost all animals and plants. Chronobiology is the systematic study of the temporal characteristics of living matter at all levels of organization, covering the study of biological rhythms and related developmental changes (REFINETTI, 2012). The seasonal rhythms of species increase individual survival and the survival of offspring when they synchronize the reproductive period with the most favourable season for offspring survival (CARVALHO-SOUSA et al., 2008). For example, in *Serinus canaria*, breeding typically occurs between the spring and summer. This breeding strategy aims to maximize favourable conditions for the offspring, with greater supplies of water and food available during these seasons characterizing the biological cycle (DAWSON, 2008).

The perception of seasons by birds involves several environmental cues that trigger physiological changes and changes to their reproductive status, and these cues include the period of daylight (photoperiod), temperature, and humidity. The most important environmental cue of seasonality is the daily duration of light (BALL & KETTERSON, 2008).

The circadian rhythm, which is generated endogenously and adjusted by environmental cues, shows persistence. It continues intermittently in an animal that has been removed from its natural environment and placed in an artificial environment with altered illumination and a constant temperature (KRONFELD-SCHOR et al., 2017). Additionally, changes to the temperature, photoperiod, oxygen level and other factors can disrupt normal circadian rhythms (CASSONE, 2014).

Several environmental factors modulate the reproductive physiology of birds, such as the environmental temperature, food availability and social interactions. Social interactions are essential for reproduction in canaries, with male presence and courtship behaviour being fundamental for the physiological adjustment of the reproductive period in the female (LESKA & DUSZA, 2007).

The decrease in daylight from autumn to winter induces animals to stay in a non-reproductive state, which is named the photosensitivity period, e.g., by causing decreases in gonad size and the production of gonadal steroids. At this stage, the animals are prepared to be stimulated to enter a reproductive state, and with the increase in the photoperiod (spring), the animals are able to reproduce. This period is known as the photostimulation period. In addition, during the time with a long photoperiod (summer), the animals are inhibited from reproducing in a period known as the photorefractory period (HURLEY et al., 2008; STOREY & NICHOLLS, 1976).

Typically, during the breeding period, each female produces four clutches, with 3 to 6 eggs per clutch, and the hatching period spans approximately 13 days. In commercial breeding, after the eggs have hatched, the young remain in the nest and are reared by their parents for approximately 30 days. After an additional 10 days, they are able to feed on their own and are placed in another cage (CANTONI, 2014).

Despite the fact that the occurrence of seasonal reproduction in birds is well known and that canary reproduction during captivity is widely performed, there are no scientific data on the manipulation of reproduction in canaries in Brazil. Thus, the aim of this study was to evaluate an artificial environment protocol in Brazil, with the photoperiod and temperature controlled, and investigates the effect of these factors on the S*erinus canaria* breeding performance.

MATERIALS AND METHODS

Animals

In total, 36 canaries (*Serinus canaria;* 18 males and 18 females) reared at a breeding establishment (Fabio Nakashima e Outro - “Sítio Paineira”, a rural company in Mogi das Cruzes, São Paulo, Brazil) and belonging to an outbred form locally called the common canary were housed under natural regimes of photoperiod and temperature.

Animals that had reached approximately 10 months of age were randomly selected one month before the natural onset time of male courtship (early July) and separated into three different groups (6 pairs/group):

EXTERNAL CONTROL – External environment control group (natural light). The animals were housed in an open room (covered and protected with a fence and mesh to prevent the entry of other animals) under natural temperature and photoperiod conditions.

ARTIFICIAL CONTROL – Internal environment control group (artificial room). The animals were housed in a closed room with the photoperiod and temperature adjusted to correspond to natural conditions.

ARTIFICIAL ALTERED – Altered internal environment group (artificial room). The animals of this group were housed concurrently with the other two groups in an artificial room but with seasonal climatic conditions opposite (different) those of by the Artificial Control. The expectation was that breeding would begin in a different seasonal period than it would in the control groups.

In all groups, one pair was housed per cage (70 cm x 27 cm x 32 cm), with water and food (seed mix and an egg-based mix) provided *ad libitum*. During all observation periods, each cage was supplied with a plastic bowl nest and fibre ropes (made from sisal) to allow for nest building, as illustrated in figure 1. The success of breeding was evaluated by the number of clutches, the number of eggs laid and the number of eggs that hatched during the breeding season.

The experiments were performed in accordance with the National Institute of Health Guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), and animals were maintained in accordance with the Brazilian Law for Procedures for Animal Scientific Use (#11794/2008). All of the experimental procedures were approved by the Institutional Ethical Committee from Mogi das Cruzes University (protocol 011/2013). All applicable institutional and/or national guidelines for the care and use of animals were followed.

Environmental data

We collected five years (2009-2013) of daily temperature (Brazilian National Institute of Meteorology) and photoperiod data (Center for Weather Forecasting and Climate Studies from Brazilian National Institute for Space Research). These environmental data were analysed and used to construct a protocol with parameters similar to those that occur naturally. The desired conditions were then established in rooms with controlled illumination time (photoperiod), air exchange, and temperature and with soundproof walls (Figure 1).

In the five-year analysis of environmental data, as expected, we observed seasonal variations in the temperature (Figure 2A) and photoperiod (Figure 2B). As expected, the lowest temperatures occur in the winter, and the greatest temperatures occur in the summer (Figure 2A). The shortest photoperiod occurs at the end of June, at 10 h 41 min:13 h 19 min (light:dark), and the longest photoperiod occurs at the end of December, at 13 h 35 min:10 h 25 min (light:dark) (Figure 2B).

Establishment of the artificial environment

Considering the limitations of the temperature control apparatus (allowing changes in 1 °C intervals), to most closely approach the natural temperature, we elected to change the temperature every 30 days (on the 21st day of each month). Thus, for the Artificial Control group, the lowest temperature (18 ºC) was established in July, and the greatest temperature (24 ºC) was established in January (Figure 3A).

For the protocol, the light period was adjusted by 12 min every 10 days (days 1, 11 and 21 of each month). To produce a photoperiod similar to that in the natural environment, the longest and shortest

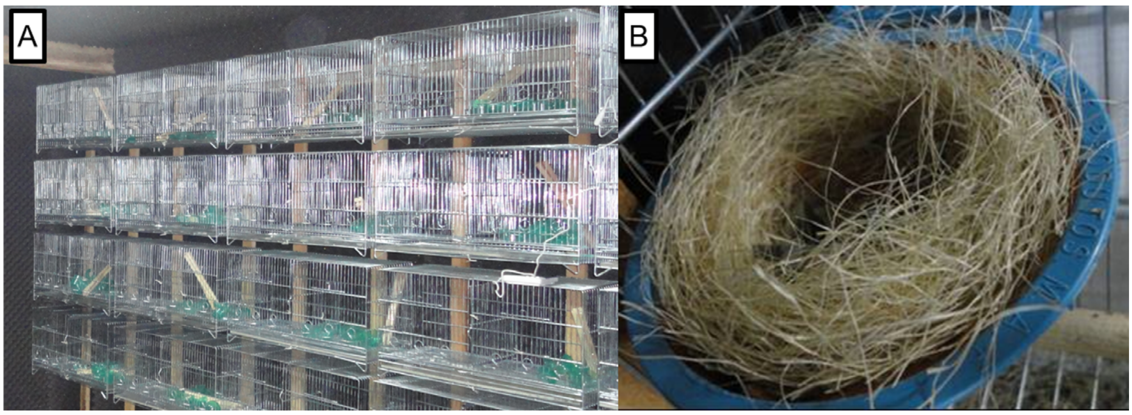

Figure 1 - Image of a representative room with a controlled artificial environment (A) and a nest constructed by a canary pair (B). Both the female and male prepare the nest to accommodate the eggs and offspring.

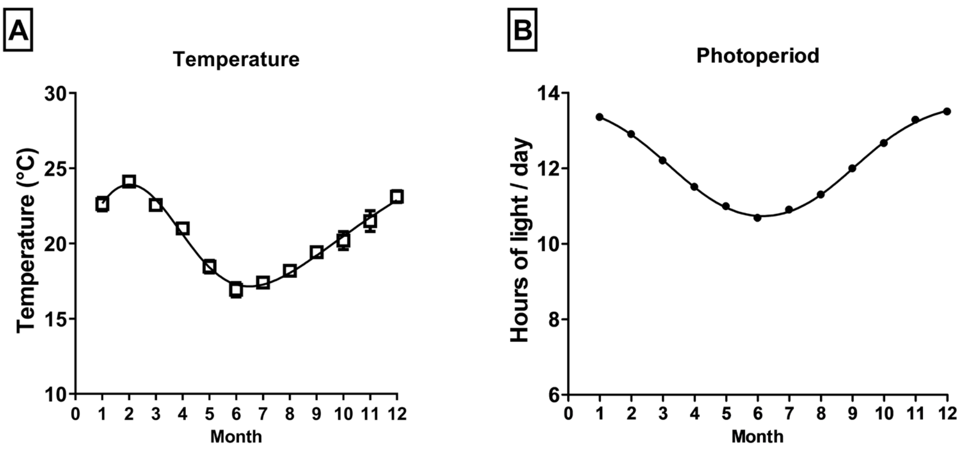

Figure 2 - The temperature (A) and photoperiod (B) recorded over 5 years in the city of São Paulo. The data are presented as the means ± SEMs of data obtained in each month over five years (2009-2013). The data were provided by the Brazilian National Institute of Meteorology (temperature) and by the Center for Weather Forecasting and Climate Studies from Brazilian National Institute for Space Research (photoperiod).

photoperiods were each maintained for 3 weeks, with the adjustment direction then reversed. For example, in the Artificial Control group, the photoperiod was increased by 12 min every ten days until the maximum photoperiod of 13 h 38 min was reached. This photoperiod was maintained for 3 weeks, and then, the daily light duration was decreased by 12 min every ten days until the minimum photoperiod of 10 h 26 min was reached. This photoperiod was then maintained for 3 weeks, after which 12 min of light were added every 10 days (Figure 3B).

In the Artificial Altered group, we implemented a protocol aimed to alter the biological clock of the animals. The animals were placed in the

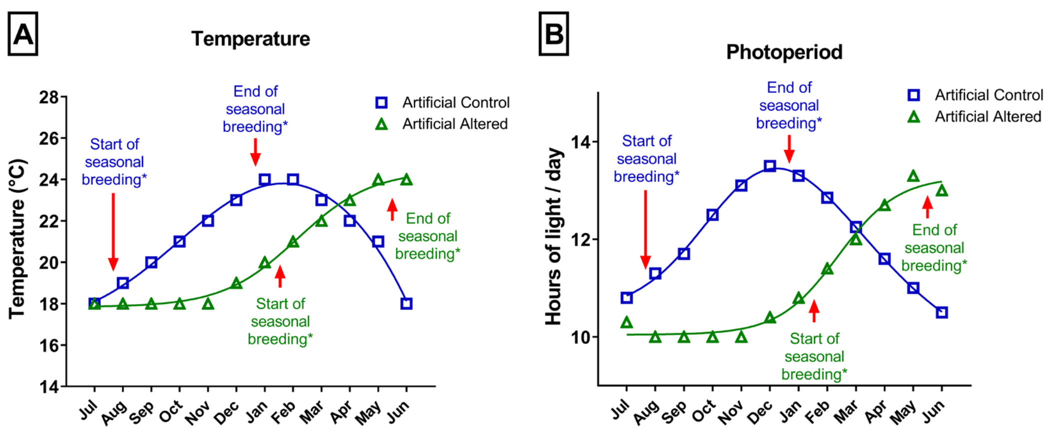

Figure 3 - Temperature protocol (A) and photoperiod (B) in the artificial rooms of the Artificial Control group (blue) and the Artificial Altered group (green) throughout the year. *, Expected start and end points of seasonal breeding.

artificial environment and maintained for 3 weeks under the same light and temperature conditions as those of Artificial Control group. After this period, the photoperiod was reduced to a short-daylight schedule (10 h 02 min:13 h 58 min, light:dark). This schedule was maintained for 4 months, and then, the light duration was gradually increased every 10 days (Figure 3B), as in the Artificial Control group. The animals were expected to start the breeding period concomitant with the end of the breeding period of the control groups (at the end of January). The temperature was also maintained at the lowest value (18 ºC) during the expected period of breeding inhibition (June-December) and then gradually increased (Figure 3A).

Statistical analyses

Analyses were performed to compare the breeding success among the groups and to determine whether canaries in controlled artificial environments can reproduce in a similar manner to their reproduction in the natural environment. The data are presented as the means ± SEM and were analysed by one-way ANOVA followed by Tukey's test. The results were considered significantly different when $P < 0.05$. All of the data were analysed with the software Graph-Pad Prism 7.0 (San Diego, CA, USA).

RESULTS

Egg laying

The canaries housed in a natural environment (External Control) or in an artificial environment with conditions similar to those of the external environment (Artificial Control) started laying eggs at the end of August, whereas the animals of the Artificial Altered group started the breeding period at the end of October (Table 1). As expected, the breeding period of the Artificial Altered group also ended at a later time than did that of the Artificial Control group. These results suggested that although the breeding period in the Artificial Altered group did not start in the expected period in January (Figure 3), we observed a delay in the onset of breeding.

In the analyses of the effectiveness of reproduction, some reproductive parameters were collected considering the total reproduction time. The experimental results indicated differences between groups in clutches laid ([$F(2, 14) = 6.012$; $P = 0.013$]), while both groups maintained in an artificial environment, Artificial Control (P=0.017) and Artificial Altered (P=0.042), laid more clutches than did the control group housed in the natural environment (External Control) (Table 2). However, despite this difference, we observed no significant difference ([$F(2, 15) = 0.503$; $P = 0.6145$]) among the groups in the total number of eggs laid in the breeding season (External Control = 20.33±2.62; Artificial Control = 23.00±3.78; Artificial Altered = 24.50±2.34) (Table 2).

Hatching

Differences were observed between the groups in the number of eggs hatched ([$F(2, 15) = 19.5$; $P = 0.0001$]). The External Control group yielded the most hatched young (11.33±1.91 birds/pair) (Table 2). In the groups housed in artificial environments, the Artificial Control group produced more hatched young than did the Artificial Altered group (6.67±1.50 and 1.83±0.91 birds/pair, respectively; P = 0.0162) (Table 2), and the differences in the percentage of eggs hatched were similar ([$F(2, 15) = 15.52$; $P = 0.0002$]) in each group (Table 2).

DISCUSSION

This research verified the possibility of manipulating the seasonal breeding of canaries through a chronobiological study. The environmental parameters of temperature and photoperiod were manipulated, as the regulation of photoperiod is the main environmental cue for the synchronization of animals and plants with seasonal changes (CASSONE, 2014). Previous protocols showed the possibility of performing seasonal manipulations (BENTLEY et al., 2003; GARCIA-FERNANDEZ et al., 2013; GOLDSMITH, 1982; HINDE et al., 1974). In the research Bentley and collaborators (BENTLEY et al., 2003), male and female canaries were housed separately under 8 h of light and 16 h of dark for 6 weeks and then immediately subjected to 18 h of light and 6 h of dark. Such manipulations could not be used for long periods in commercial breeding, as

Table 1 - Beginning and end of the egg laying period. The data are presented as the mean dates for each group (n= 6 pairs/group).

GROUP	START	END
External Control	30/08	03/02
Artificial Control	17/08	06/02
Artificial Altered	29/10	22/04

Table 2 - Breeding data per pair. The data are presented as the means ± SEMs.

Reproduction Parameters/pair	External Control	Artificial Control	Artificial Altered
Number of clutches laid	4.67±0.49	6.83±0.48*	6.60±0.51*
Total number of eggs	20.33±2.62	23.00±3.78	24.5±2.33
Number of eggs hatched	11.33±1.91	6.67±1.50*	1.83±0.91*,#
Percentage of eggs hatched	56.87±8.85	31.13±6.35*	5.86±2.65*,#

*, $P<0.05$ compared with the External Control group. #, $P<0.05$ compared with the Artificial Control group.

such abrupt photoperiod changes might have negative effects over the long term, causing dysfunctions of the endogenous clock and leading to several problems in the animal health (STEVENSON et al., 2015). Therefore, the protocols developed in the present research were aimed to mimick the gradual changes in temperature and photoperiod that occur in the natural environment (according to the limitations of the devices) to reduce the impacts of the artificial environment and perform a chronobiological study with similar conditions to those found in nature.

As previously shown, the onset of canary reproduction depends directly on the increase in the photoperiod, which results in physiological and hormonal changes that culminate in the breeding period (BENTLEY et al., 2003). In this study, we attempted to inhibit the natural breeding period in the Artificial Altered group. Initial inhibition was observed under the conditions of low temperatures and a short photoperiod; however, even under these conditions, the canaries of the Artificial Altered group converted to an active breeding status after 4 months.

Considering that the initial goal of this project was to verify the influences of photoperiod and temperature on the reproductive status of animals in this protocol, the members of a pair were consistently housed together during the experiments, and a nest and nesting material were consistently provided during the experiments in all groups. These practices reduced the number of variables; however, they might have provided environmental cues of the breeding period to the animals. Males are more sensitive to photoperiod than are females in terms of gonad stimulation, and male courtship behaviour can induce the appropriate responses of reproductive organs in females and stimulate other males to prepare for reproductive competition (BENTLEY et al., 2000; LEITNER et al., 2006). Although, each experimental room was soundproofed, the vocalizations of males within a room might have stimulated reproductive behaviour in other males in the room. The exposure of a female to the specific vocalizations of her partner can lead to increased reproductive physiological function and changes in behaviour, for example, inducing nesting behaviour (BENTLEY et al., 2000; HINDE & STEEL, 1976; LEITNER et al., 2006; TRAMONTIN et al., 1999).

In the Artificial Control group, which was housed in an artificial setting that mimicked the natural environment, there was a greater number of eggs laid than that observed in the animals housed in the natural environment. Our hypothesis is that this finding is due to the greater stability of the temperature and relative humidity of the air than that in the natural environment, with fewer oscillations, as we recorded large variations in temperature and relative humidity in the natural environment (data not shown), in addition to the normal diurnal variation. However, in the artificial environment, the temperature changes were well controlled, and the relative humidity did not change significantly (remaining at approximately 60-70%). Climatic variations are related to reproductive success in several groups of animals (NISHIWAKI-OHKAWA & YOSHIMURA, 2016), which is promoted by changes in seasonality, such as in temperature, humidity and food availability (DAWSON, 2008).

The number of eggs hatched was greater in the External Control (outdoor) group than in the groups housed in artificial rooms (inversely to the number of eggs laid). These results indicated that the natural oscillations in environmental conditions might affect hatching success. In the artificial-room groups, the number of hatched young was greater in the Artificial Control group (with conditions mimicking those of the natural environment) than in the Artificial Altered group. These results could be attributed to the fact that the onset of breeding in the Artificial Altered group occurred under low-temperature and low-luminosity conditions, which we hypothesized would not favour hatching.

Another aspect to consider in this study is that only young birds of early sexual maturity (1 year of age), that had never reproduced, were used. Age is an important factor in the mating of *Serinus canaria* and affects the sexual response (COELHO, 2004). Although, all the animals used in this study were of the same age, the low egg hatching success in the groups, particularly in the externally housed control group (-60%), might have been due pair inexperience in behaviours such as nest preparation and egg brooding. In addition, hormonal oscillations are responsible for both the maturation of the gonads and the parental behaviours required at each breeding phase, such as nest building, egg incubation and feeding of the young (GOLDSMITH, 1982). These behaviours might not have been well developed in the young canaries used in this research. However, notably, regardless of the inexperience of the animals, similar hatching success rates (60-70%) were observed in previous studies (HINDE, 1959; LEITNER et al., 2006; SCHWABL, 1996).

The availability of food can also be important in the breeding period because it can act as an environmental cue. In birds, several species have shown relationships between breeding success and the abundance or diversity of food, with specific effects on the number of eggs laid (DAWSON, 2008; HAU, 2001). In the present study, only the photoperiod and temperature were used as environmental cues of the breeding period. Furthermore, the change in the availability of food in captivity might be used as a reinforcer of seasonality (HAHN & MACDOUGALL-SHACKLETON, 2008; HAU, 2001; TAYLOR et al., 1994).

The data presented herein should contribute significantly to other studies of bird physiology, particularly those involving chronobiological aspects. The protocols of temperature and photoperiod manipulation described here can be performed with lower adverse effects on organism physiology relative to those of previous protocols that entail abrupt changes in environmental parameters.

CONCLUSION

In the present research, we observe that canaries from the Artificial Control group, which was composed of animals housed in an internal room that mimicked the natural environment, had a greater number of eggs laid than that observed in the animals housed in the natural environment; however, the number of eggs hatched was greater in the External Control group than in the groups housed in artificial rooms (inversely related to the number of eggs laid). Furthermore, in the artificial-room groups, the number of hatched young was greater in the Artificial Control group than in the Artificial Altered group.

Regarding the manipulation of luminosity to inhibit the natural breeding period in the Artificial Altered group, the protocol was partially successful and was observed under the conditions of a low temperature and short photoperiod; nonetheless, the canaries started breeding after 4 months, even under a photoperiod for which this was not expected. Therefore, although, further research is needed, the present research provided more data to assist in the development of protocols that entail gradual changes in environmental conditions, which should have low negative impacts on the reproduction of captive animals.

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BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

All of the experimental procedures were approved by the Institutional Ethical Committee from Mogi das Cruzes University (protocol 011/2013). All applicable institutional and/or national guidelines for the care and use of animals were followed.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest. The manuscript does not involve clinical studies or contain patient data.

AUTHORS' CONTRIBUTIONS

EKT conceived, designed and performed the experiments, analysed the data and wrote the manuscript. RI, PCLM and FN designed and performed the experiments. PHLM analysed the data and wrote the manuscript. All authors read and approved the final manuscript.

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